Practitioner's Docket No. 741135-000010 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	SIFFERT, W.	Group:	Not yet assigned
Application No.:	Not yet assigned	Examiner:	Not yet assigned
Filed:	Herewith		

(Continuation of 09/180,783 – Filed: 17 March 1999)

For: THE USE OF A GENETIC MODIFICATION IN THE GENE FOR HUMAN G PROTEIN β3 SUBUNIT FOR THE DIAGNOSIS OF DISEASES

CERTIFICATE OF MAILING

I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as Express Mail Label No. EL565099793US in an envelope addressed to Box Patent Application Assistant Commissioner of Patents, Washington, D.C. 20231.

Date: 16 April 2001

Nicole M. Gignac

Box Patent Application
Assistant Commissioner for Patents
Washington D.C. 20231

PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in the U.S. Patent and Trademark Office concurrently with a Continuation Patent Application in the above-referenced matter.

Preliminary to calculation of the filing fee and examination on the merits, please amend the application identified in caption as follows:

IN THE CROSS-REFERENCE PARAGRAPH:

Please insert the following paragraph as the first paragraph on the first page in the application:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of U.S. Patent Application Serial No. 09/180,783 filed on November 16, 1998, the content of which is relied upon and incorporated herein by reference in its entirety, and the benefit of priority under 37 U.S.C. § 120 is hereby claimed.

IN THE SPECIFICATION:

Page 1, please replace the first paragraph at Line 5 with the following paragraph:

BACKGROUND OF THE INVENTION:

(i) Field of the Invention

The present invention relates to a method for the diagnosis of diseases by genetic analysis, in particular the analysis of genes for subunits of the human guanine nucleotide-binding proteins (G proteins).

Page 1, please replace the second paragraph at Line 10 with the following paragraph:

(ii) Description of the Related Art

Heterotrimeric guanine nucleotide-binding proteins (G proteins) have an outstanding importance in intracellular signal transduction. They mediate the relaying of extracellular signals after stimulation of hormone receptors and other receptors which undergo a conformational change after receptor activation. This leads to activation of G proteins which may subsequently activate or inhibit intracellular effectors (e.g. ion channels, enzymes). Heterotrimeric G proteins consist of three subunits, the α, β and γ subunits. To date, several different α subunits, 5 β subunits and about 12 γ subunits have been detected by biochemical and molecular biological methods

(Birnbaumer, L. and Birnbaumer, M. Signal transduction by G proteins: 1994 edition. *J. Recept. Res.* 15:213-252, 1995; Offermanns, S. and Schultz, G. Complex information processing by the transmembrane signaling system involving G proteins. *Naunyn Schmiedebergs Arch. Pharmacol.* 350:329-338, 1994; Nürnberg, B., Gudermann, T., and Schultz, G. Receptors and G proteins as primary components of transmembrane signal transduction. Part 2. G proteins: structure and function. *J. Mol. Med.* 73:123-132, 1995; Neer, E.J. Heterotrimeric G proteins: Organizers of Transmembrane Signals. *Cell* 80:249-257, 1995; Rens-Domiano, S. and Hamm, H.E. Structural and functional relationships of heterotrimeric G-proteins. *FASEB J.* 9:1059-1066, 1995).

Page 1, please replace the fourth paragraph at Line 39 with the following paragraph:

SUMMARY OF THE INVENTION:

We have found that a genetic modification in the gene for human G protein β3 subunits is suitable for the diagnosis of diseases. This genetic modification is particularly suitable for establishing the risk of developing a disorder associated with G protein dysregulation.

Page 2, please replace the second paragraph at Line 6 with the following paragraph:

BRIEF DESCRIPTION OF THE DRAWING:

The figure depicts a comparison of genes from normotensives and hypertensives by restriction enzyme analysis.

Page 2, please insert the following as paragraph three (following the preceding paragraph):

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS:

The genetic modification which has been found is located in the gene for human G protein β3 subunit. This gene has been described by Levine et al. (Proc. Natl. Acad. Sci USA, 87, (1990)

2329-2333). The coding region has an Ser codon (TCC) at position 275, while subjects with an increased risk of a disease associated with G protein dysregulation have the codon TCT, which likewise codes for Ser, at this position. The genetic modification is a base substitution at position 825 in which a cytosine (C) is replaced by thymine (T). However, this base exchange is "silent" at the amino-acid level, ie. It does not lead to incorporation of a different amino acid at this position. The sequence found in subjects with an increased risk of disease is depicted in SEQ ID NO:1 in the sequence listing.

IN THE CLAIMS:

Please cancel claims 2 -12 and add new claims 13-31 as follows:

13. A method of diagnosing a disease comprising determining the presence of a genetic modification in a gene obtained from a subject which encodes a human G protein β_3 subunit.

14. The method as claimed in Claim 13, wherein said disease is a disorder associated with G protein dysregulation.

15. The method as claimed in Claim 13, wherein said gene which encodes a human G protein β_3 subunit is the gene of SEQ ID NO: 1.

16. The method as claimed in Claim 15, wherein the genetic modification is in the codon for amino acid 275 in SEQ ID NO: 1.

17. The method as claimed in Claim 16, wherein the genetic modification is a substitution of cytosine by thymine at position 825 in SEQ ID NO: 1.

18. The method as claimed in Claim 14, wherein the disorder is a cardiovascular disease, a metabolic disturbance or an immunological disease.

19. The method as claimed in Claim 14, wherein the disorder is hypertension.

20. A method for establishing the relative risk of developing a disorder associated with G protein dysregulation for a subject, comprising the steps of:

(I) determining the presence of a genetic modification in a gene obtained from a subject which encodes a human G protein β_3 subunit;

(II) in the event the presence of a genetic modification is determined, assigning the subject an increased risk of disease.

21. The method as claimed in Claim 20, comprising comparing said gene obtained from a subject which encodes a human G protein β_3 subunit to the gene sequence of SEQ ID NO: 1.

22. The method as claimed in Claim 21, wherein the genetic modification which is determined is the presence of a thymine (T) at position 825 in the gene obtained from the subject.

23. The method as claimed in Claim 20, wherein the presence of a genetic modification in the gene obtained from a subject is determined by sequencing.

24. The method as claimed in Claim 23, further comprising the step of amplifying the gene obtained from the subject before sequencing.

25. The method as claimed in Claim 23, wherein a section the gene from the host corresponding to position 825 in the gene of SEQ ID NO: 1 is amplified.

26. The method as claimed in Claim 20, wherein the presence of a genetic modification in the gene obtained from the subject is determined by hybridization.

27. The method as claimed in Claim 20, wherein the presence of a genetic modification in the gene obtained from the subject is determined by cleavage using a restriction enzyme.

28. The method as claimed in Claim 27, wherein the restriction enzyme is Dsa I.

29. A non-human transgenic animal comprising a gene which encodes a modified human G protein β_3 subunit.

30. The non-human transgenic animal as claimed in Claim 29, which encodes a modified human G protein β_3 subunit of SEQ ID NO: 1.

31. The non-human transgenic animal as claimed in Claim 30, wherein said modified human G protein β_3 subunit includes a substitution of cytosine with thymine at position 825.

IN THE SEQUENCE LISTING:

Please delete the Sequence Listing on pages 7-9 of the application, and insert the enclosed substitute Sequence Listing into the application.

IN THE ABSTRACT:

Please delete the abstract of the disclosure in its entirety and insert thereafter the following replacement:

A method of diagnosing a disease comprising determining the presence of a genetic modification in a gene obtained from a subject which encodes a human G protein β_3 subunit. Also disclosed is a method for establishing the relative risk of developing a disorder associated with G protein dysregulation.

REMARKS

By the present Preliminary Amendment, Applicants have added the headings suggested by the U.S. Patent and Trademark Office at the appropriate places in the specification. Applicants have amended the claims to better conform them with U.S. practices.

Applicant previously submitted an Amendment to the Sequence Listing in the parent application. In accordance with 37 C.F.R. §§ 1.821 – 1.825, a Substitute Sequence Listing was submitted. Accordingly, Applicant respectfully submits herewith a Transfer Sequence Listing, stating that the content of the paper and computer readable copies of the sequence listing are respectively the same as the those in the Substitute Sequence Listing submitted on July 10, 2000 in the parent application.

In the event that there are any questions relating to this Amendment or to the application in general, it would be appreciated if the Examiner would contact the undersigned attorney concerning such questions so that prosecution of this application can be expedited.

Entry of the foregoing and prompt and favorable consideration of the subject application on the merits are respectfully requested.

Date: 16 April 2001

Respectfully submitted,

Customer No.: 26770

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VERSION WITH MARKINGS TO SHOW CHANGES MADE TO THE SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

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"silent" at the amino-acid level, ie. it does not lead to incorporation of a different amino acid at this position. The sequence found in subjects with an increased risk of disease is depicted in SEQ ID NO: 1 in the sequence listing.

ABSTRACT

~~The present invention relates to the use of a genetic modification in the gene for human G protein β3 subunit for the diagnosis of diseases.~~

A method of diagnosing a disease comprising determining the presence of a genetic modification in a gene obtained from a subject which encodes a human G protein β3 subunit. Also disclosed is a method for establishing the relative risk of developing a disorder associated with G protein dysregulation.